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The relevance of zinc determination in amniotic fluid
1st Communication: Development of the technique and comparative determination of zinc and protein

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1 Introduction

Zinc (Zn), which is a component of many important enzymes, is one of the trace elements required by humans and other mammals. The total amount in the human body lies between 1.4 and 2.3 g [14], of which 95% to 98% is complexed to proteins [12, 14]. The biological availability seems to depend on the nature of the binding to the protein. Zinc deficiency leads to a reduction in DNA and RNA synthesis [1], so that the synthesis of amino acids and proteins is also zinc dependent. Hypoalbuminemias caused by zinc deficiency lead to dwarfism [9]. A decrease in the content of albumin and zinc in the maternal blood was observed in EPH gestosis [3].

Zinc is also important for the antibacterial activity of the amniotic fluid (AF). It was shown that two components, namely zinc and a short-chain protein, are responsible for the bacteriostatic properties of amniotic fluid [15, 16]. The levels of zinc in serum have been measured by many groups, of which only a few can be mentioned here [3, 6, 7, 11, 12, 20, 21]. The normal range lies about 1 μg Zn/ml serum. In contrast, the few published values for the zinc content of amniotic fluid are inexact [10, 15].

The pathological conditions caused in humans by changes in the zinc balance can be divided into four groups. The first comprises genetically caused disturbances, including acrodermatitis enteropathica and familial hyperzincemia. The second group is caused by nutritional deficiency. In Egypt and Iran, small stature due to zinc deficiency was observed among population groups whose diet is one-sided in favor of too much white bread. One of the causative factors is the large amounts of phytic acids ingested, which form complexes with zinc salts and interfere with resorption through the intestinal wall [5]. In these countries, the incidence of neurological damage among infants is very high [17]. It is also known that the maternal serum zinc levels drop sharply, which indicates an increased zinc requirement. Experiments with animals on zinc-deficient diets have shown that they regularly abort or produce seriously deformed fetuses and newborns. The birth process is delayed. The third group consists of medication-induced disturbances. Diuretics or drugs inducing tissue degradation lower the serum zinc level. The same applies to the effects of corticoids [2]. Zinc metabolism is also affected by other elements, like calcium, cadmium and copper [18].

Disease-induced conditions make up the fourth group. It includes besides the EPH gestosis and acrodermatitis enteropathica already mentioned, bronchial carcinoma, alcoholic cirrhosis of the liver and diabetes mellitus [14].

Adequate serum zinc levels are important in the healing of skin defects, in particular for ulcus cruris. Some of the deficiency symdromes listed can be improved by zinc therapy (about 30 mg Zn/day) [4, 5, 13].

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2 Objectives

From the facts discussed above, it can be seen that information on the Zn metabolism during the perinatal period is important, and the analysis of the amniotic fluid is a good way to obtain such information. A special method for determining zinc in the amniotic fluid had to be developed and checked for possible sources of error. Norms for the level at each week of gestation had to be established by examination of a large number of amniotic fluid samples. The work had to include comparisons of zinc and protein levels, since these are closely correlated, due to the binding of zinc to protein. For this purpose it was necessary to develop a rapid and simple method of protein determination which was suitable for the much lower concentrations in amniotic fluid than in serum. Due to the zinc-dependence of protein synthesis mentioned above, it was important to determine whether the protein and zinc levels develop comparably during the pregnancy, and whether either of the parameters was age-dependent. The results are to be used in a further project [8] to determine whether defined perinatal syndromes cause a disturbance in the zinc balance.

3 Material and methods

More than 600 deep-frozen (–20°C) amniotic fluid samples were available for the experiment. The samples were obtained by amniocentesis and by puncture of the membrane during delivery. Only the samples from normal pregnancies were used for the determination of control values.

3.1 Determination of zinc

A PERKIN ELMER atomic absorption spectrophotometer 403 was used for measurements. Light source: Zn hollow cathode lamp; wavelength for measurements: 213.9 nm; lamp current: 15 mA; slit width: 0.7 Å; fuel gas: air/acetylene. Standard solution: A standard solution was made by dissolving 500 mg granulated analysis-grade zinc (99.9%, MERCK, Darmstadt, Germany) in 20 ml 16% hydrochloric acid (HCl) and filling to 1 liter with 1N HCl. 2.0 ml of this solution and 500 ml 6% Macrodex made up to 1000 ml gave the standard solution of 1.00 µg Zn/ml. The zinc contents of all analysis-grade reagents used, such as lanthanum nitrate, sodium phosphate, and solvents like HCl, Macrodex and double-distilled water were checked.

Accuracy of measurement: The results of the determination on a dilution series of the Zn standard are shown in Fig. 1. The calibration curve shows a linear dependence of the measured zinc content on the true content of the samples in the main region of measurement up to 1.4 µg Zn/ml. An amniotic fluid pool with known zinc content was used to test the applicability of the method to samples of amniotic fluid. The relative standard deviation of the individual measurements was 2.1%. When known amounts of zinc were added to the amniotic fluid pool (AF addition curve) the recovery rate was 92.6% and the measured value was linearly dependent on the Zn amount added (Fig. 2). This experiment showed that the

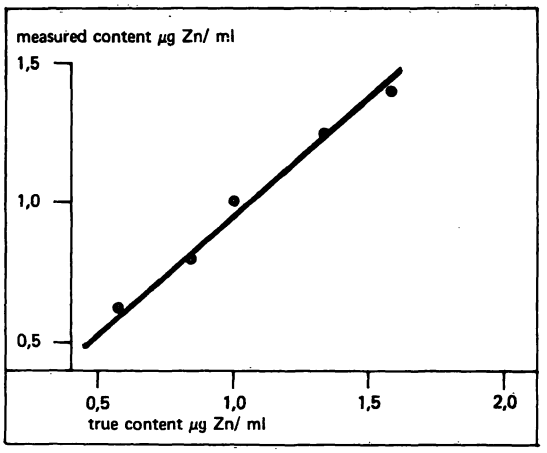

Fig. 1. Experiment to test the linearity of the calibration curve with a Zn standard.

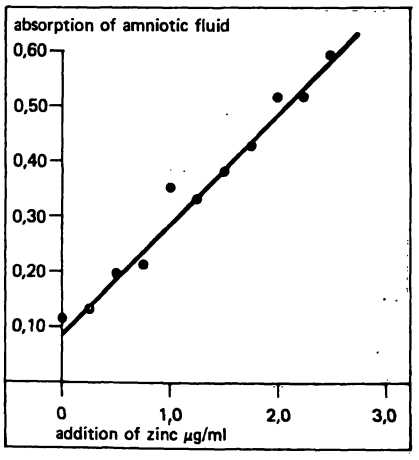

Fig. 2. Amniotic fluid: calibration curve obtained with the addition technique.

amniotic fluid has no effect on the linearity of the calibration curve, up to a concentration of 2.5 μg Zn/ml. Due to the zinc present in the amniotic fluid, the curve naturally does not go through zero. The addition method shown in Fig. 2 is a reliable technique for checking for physical and chemical interferences. Unspecific light losses, caused by molecular absorption or scattering by solid or liquid particles, which are plentiful in amniotic fluid, as well as too low a vaporization temperature or matrix effects, which lead to incomplete atomization, can be recognized. These results show that both the proportionality between absorption and concentration and the accuracy of the values obtained are adequate. Dilution of the amniotic fluid samples, as is required for serum samples, is thus unnecessary. The additional measurement with deuterium background compensation gave the same results. It is thus certain that a complete, high-temperature vaporization has occurred. The relative standard deviation of the individual measurements in the concentration range around 1 μg Zn/ml AF is about 1% and the detection limit is about 0.02 μg Zn/ml AF.

To determine the precision of the method, the chemical interferences as well as the physical had to be evaluated. The former arise through the influence of foreign anions or cations on the atomization equilibrium in the sample. In amniotic fluid such effects could be caused in particular by phosphates, which affect the measurement by forming insoluble zinc phosphates. This can be prevented by addition of lanthanum salts. The corresponding experiments showed that addition of 5 or 10 mg lanthanum (as nitrate) to amniotic fluid samples of known zinc content produced no measurable change in the signal. It has thus been established that chemical interference is not a significant source of error and that it is acceptable and practical to use undiluted amniotic fluid without additions for the measurements. This means that large numbers of samples can be analyzed in a relatively short time. If one allows 15 min for the preparation and calibration of the apparatus, then a time of about 2 min per sample is needed for larger numbers of samples.

The atomic absorption spectrophotometer was calibrated with the standard solution of 1.00 μg Zn/ml. The amniotic fluid samples were thawed and, when necessary, centrifuged. The instrument was recalibrated after every 20 samples. The results were averaged from at least 10 measurements, which require about 2 ml amniotic fluid. If larger volumes of amniotic fluid were available, the average was taken of up to 100 individual measurements. The results were subjected to statistical analysis by the U-test of MANN, WHITNEY and WILCOXON.

3.2 Determination of total protein

Measurements were made on an EPPENDORF photometer with a mercury filter, 546 nm. Total protein (TP) was determined by a Biuret method modified for amniotic fluid (Testkombination Nr. 15899 from BOEHRINGER, Mannheim, Germany). The amounts of reactants had to be modified, as they were intended for serum, which has a much higher protein concentration than amniotic fluid. For a dilution of 1:10, corresponding to 600 mg TP/100 ml, the absorption was linearly dependent on the protein content of the samples in the range of 100 to 750 mg TP/100 ml AF (Fig. 3).
Tab. I gives the mixtures (μl) used for the various measurements.

Tab. I. Mixing table for reactants.

	Baseline	Control	Standard	Analysis
Water	100	–	–	–
Control reagent	–	1000	–	–
Biuret soln.	1000	–	1000	1000
Standard 1:10	–	–	100	–
Amniotic fluid	–	100	–	100

The relative standard deviation in the concentration range 600 mg TP/100 ml was 4%, and for the range 200 mg TP/100 ml, 7%. Measurement against the control serum Precilip 314 (BOEHRINGER,

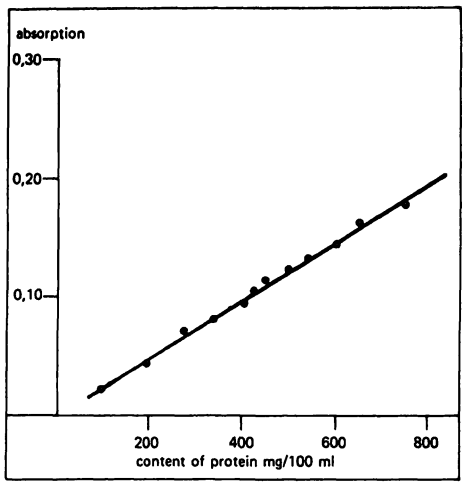

Fig. 3. Test of the linearity of the calibration curve with a protein standard.

Mannheim, Germany) gave a recovery rate of 97.3%, while with Kontrollogen KL 405 c (BEHRING-Werke, Marburg, Germany) the recovery rate was 94.8%. An experiment analogous to the Zn addition curve with increasing protein concentrations gave recovery rates of 101.0%. The limit of concentration of this modified method is about 50 mg TP/100 ml AF. Since the amniotic fluid samples examined in the present experiments had distinctly higher protein contents, the method is applicable for protein determination in amniotic fluid.

A 1:10 diluted standard was used in this series of measurements. The AF samples were allowed to thaw for 20 min, then shaken and finally centrifuged for 10 min at 3000 rpm. Samples which could not be clarified by this treatment were discarded. The control reaction serves to eliminate errors with green and hemorrhagic amniotic fluid samples. The samples were kept at 25°C during the measurement.

4 Results

The results are mainly presented in terms of the median values and of 10th percentiles, since these values give a more accurate picture of the relationships than the means. The reason is that one finds higher zinc contents in a small percentage of the amniotic fluid samples. The distribution is thus not a symmetric one. An indication of a possible source of contamination was given by a group of 30 AF samples which, unlike the others, were not kept in polyethylene tubes, but in glass tubes with rubber stoppers. In these samples, extremely high zinc contents were measured; the values were in some cases over 4 μg Zn/ml AF, and averaged 2 to 3 μg Zn/ml AF. To decide whether the zinc contamination comes from the glass or the rubber stoppers, the appropriate storage experiments were run on amniotic fluid with known Zn content. The experiments showed that the contamination comes from both the glass and the stoppers. In the evaluation of the results, only samples which had been stored in polyethylene tubes were included. These findings confirm the importance of testing reagents and materials used in Zn determination for contamination or the possible release of zinc.

Fig. 4 shows the average zinc content of the amniotic fluid as a function of the age of the mother. The median values of Zn content at term change only slightly with increasing age of the mother. The decrease is not statistically significant ($p = 0.31$). The means, which have been plotted for comparison, are significantly higher, for the reasons discussed. Since 531 samples were tested in this experiment, the statistical confidence in the results is adequate. The 90th percentiles, which are not plotted in Fig. 4, lay between 0.38 and 0.68 μg Zn/ml AF.

The zinc contents of 440 amniotic fluid samples are plotted against the gestational week (WG) (Fig. 5). After the 37th week, there is a sharp increase in the zinc values. The median values at term are twice as high as those for the middle trimenon. In the 42nd WG there is a further large increase in the zinc values, which are then three times the starting value. The increase after the 37th WG is highly significant by the U-test with $p < 0.001$. The 10th and 90th percentiles show the same steep increase. Fig. 6 and 7 show some of our results on the AF protein content at term. Fig. 6 shows the protein content as a function of the age of the mother in 300 samples. In contrast to zinc, there is a certain dependence on age here, with a maximum in very young mothers and in the range of the 30th to 34th year. The U-test indicates a significant difference with $p < 0.01$ for the age groups 19–24 years ($n = 102$) and 30–34 years ($n = 85$). Fig. 7, based on 192 samples,

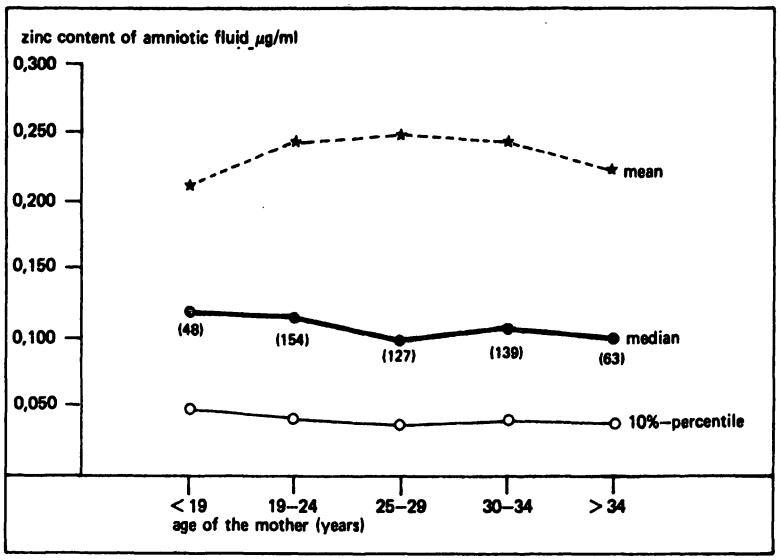

Fig. 4. Zinc content of the amniotic fluid as a function of the mother's age. ( ) = number of samples.

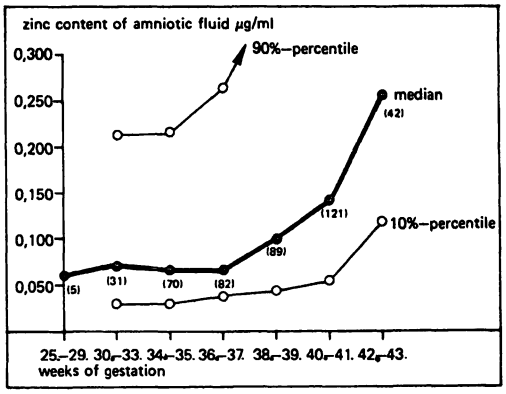

Fig. 5. Zinc content of the amniotic fluid as a function of the week of gestation. ( ) = number of samples.

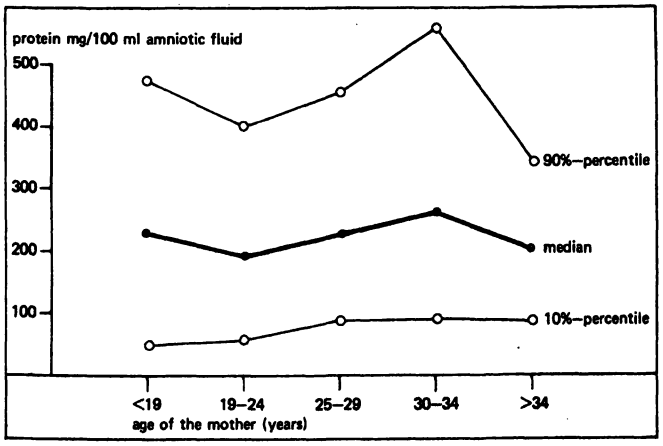

Fig. 6. Protein content of the amniotic fluid as a function of the mother's age (n = 300).

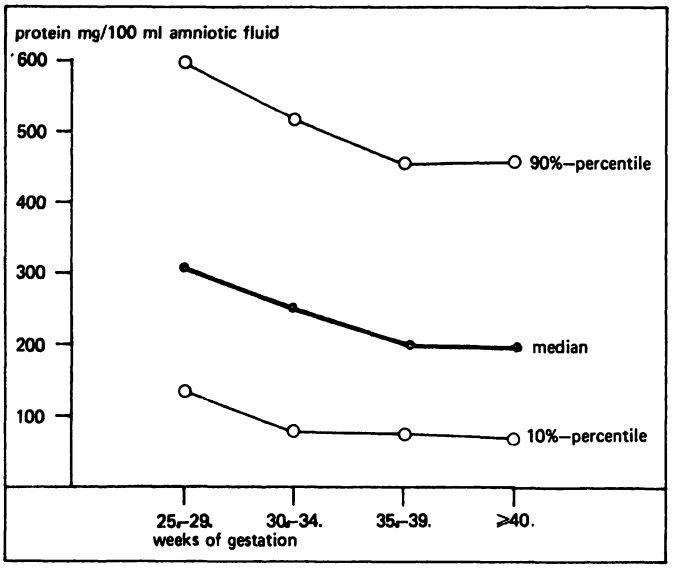

Fig. 7. Protein content of the amniotic fluid as a function of the week of gestation (n = 192).

shows the decrease in the protein content of the amniotic fluid after the 25th WG. The decrease up to the 39th week is significant ($p < 0.05$). From the 40th WG on, the values remain constant.

In order to be certain that there are no sex-specific differences in the zinc and protein concentrations, a comparative analysis was carried out. 180 amniotic fluid samples from male fetuses and 204 amniotic fluid samples from femal fetuses had the same median zinc values. The median

protein content of 165 samples from male fetuses was 236 mg TP/100 ml AF, while that of 184 female fetuses was 264 mg TP/100 ml AF. Here too, the differences were small and not statistically significant.

5 Discussion

The determination of zinc by atomic absorption spectrophotometry is a rapid procedure with a high degree of sensitivity, precision and accuracy. Since only a few minutes per determination are needed, and since the samples do not have to be specially prepared, aside possibly from centrifugation (vernix, meconium), it is possible to examine a large number of AF samples in a short time. The procedure can be largely automatated, so that it can also be carried out by technicians. As with all analyses of trace elements, however, great care must be taken to exclude possible sources of contamination. This holds especially for the equipment used to withdraw the samples and for their storage. Rubber stoppers and glass tubes cannot be used. The reagents must be tested for their freedom from zinc.

In contrast to the protein content, the **zinc concentration increases in the course of the pregnancy.** The increase after the 37th WG is significant (Fig. 5). With all due caution, one may conclude that this is an indication of an increased rate of protein synthesis, due to the large absolute increase in fetal weight in the last third of pregnancy. Thus the zinc value could be a better parameter for the fetal development than the protein concentration, which is not very useful, among other things because of the wide range of normal values. According to SUTCLIFFE and BROCK [19], the protein content sinks to about half its previous value between the 25th and 32nd WG, and then remains nearly constant until the end of the pregnancy. Our studies produced similar results (Fig. 7). In addition to this, we found, as already mentioned, a dependence of the protein values on the age of the mother. For these reasons, the protein content of the amniotic fluid is an indication neither of the age of the pregnancy nor of an unimpaired fetal development.

Since the above described studies were intended to establish normal values, only amniotic fluid samples from non-pathological, unremarkable pregnancies were used. These results will be compared with those from amniotic fluid from pathological pregnancies [8]. In this way it will be determined whether the measurement of zinc in amniotic fluid can be used for diagnosis and supervision of fetal deficiency development. This assumption appears not to be too far wrong, because studies on hypotrophic fetuses have shown that they have distinctly lower amniotic fluid zinc values [8].

Summary

Zinc is one of the essential trace elements, both as a component of many important enzymes and due to its role in protein biosynthesis. Deficiency can be detected in both humans and animals. Hypoalbuminemias caused by zinc deficiency lead to dwarfism. In humans, the causes of hypozinkemias were found to be genetic factors, nutrition, medication and disease. In EPH gestosis, a decrease in the albumin and zinc contents of the maternal blood was observed. Furthermore, zinc is important for the antibacterial activity of the amniotic fluid (AF). In countries where zinc deficiency causes small stature, the rate of neurological damage among infants is also very high. In animal studies of artifically induced zinc deficiency, the animals usually abort or the young are badly deformed; the delivery is delayed.

In light of the apparent significance of zinc metabolism in the perinatal period, it seemed important to examine thoroughly the amniotic fluid, which, in contrast to the serum, has been only slightly studied. Since zinc and protein metabolism are closely correlated, the study had to include comparative protein measurements. Adequate methods for the determination of both parameters were developed. Zinc was determined by means of atomic absorption spectrophotometry. Physical and chemical interferences were eliminated, and the possibility of contamination from external sources was examined. The relative standard deviation of the individual measurement is about 1% in the concentration range about 1 μg Zn/ml AF, and the limit of detection is about 0.02 μg Zn/ml AF. The recovery rate with a linear calibration curve is 92.6%. The time required per determination is a few minutes, since aside from possible centrifugation, the samples require no preparation before measurement. The technique can be largely automated and carried out by technicians.

Total protein (TP) is measured by a Biuret method adapted for amniotic fluid. Due to the low protein concentration in AF, the amounts of reagents from the BOEHRINGER, Mannheim kit, which is meant for serum, were changed correspondingly. The relative

standard deviation is about 7% for protein concentrations of about 200 to 300 mg/100 ml AF, the limit of detection is about 50 mg TP/100 ml AF, and the recovery rate, measured against two control sera, was 97.3 or 94.8%. More than 600 AF samples were available for this study, and of them, only those from normal pregnancies were used to establish the normal values.

The zinc and protein contents of the AF were plotted against the age of the mother or against the week of pregnancy, in order to discover possible correlations. The zinc content does not depend on the age of the mother, but does depend on the week of gestation (WG). **In the third trimenon there is a highly significant (U-test, $p < 0.001$) rise in the zinc content, from about 0.06 µg Zn/ml AF to 0.14 µg Zn/ml AF.** Thus the median values in the last trimenon have doubled with respect to the middle trimenon, and reach 0.22 µg Zn/ml AF in the 42nd WG.

The protein content is dependent on the age of the mother, to a certain extent, with increased values in very young mothers and in the range of the 30th to 34th year. The protein content decreases in the 3rd trimenon. The sex of the fetus made no difference either on the zinc or the protein level.

Distinctly lower zinc values were found in the amniotic fluid from hypotrophic fetuses, as will be reported in a following paper [8].

It can be concluded that the increase in the zinc content of the AF is an expression of a normal pregnancy. Conversely, a decrease in the amniotic fluid zinc content can serve as an indicator of a pathological development. The determination of the AF zinc content thus appears superior to the determination of protein content in several respects, and unlike the latter, can serve as an indicator of normal fetal development.

Keywords: Amniotic fluid, fetal supervision, proteins, risk pregnancy, trace elements, zinc.

Zusammenfassung

Die Bedeutung der Zinkbestimmung im Fruchtwasser. I. Mitteilung: Methodenentwicklung und vergleichende Bestimmung von Zink und Eiweiß

Als Bestandteil vieler wichtiger Enzyme und wegen seiner Rolle bei der Proteinbiosynthese gehört Zink zu den essentiellen Spurenelementen. Mangelzustände konnten bei Mensch und Tier nachgewiesen werden. Die durch Zinkmangel hervorgerufenen Hypoalbuminämien führen zu Zwergwuchs. Als Ursachen für Hypozinkämien konnten beim Menschen genetische Faktoren, alimentäre, medikamentös verursachte und krankheitsbedingte Störungen nachgewiesen werden. Bei EPH-Gestosen konnte ein Abfall des Albumingehaltes und des Zinkgehaltes im mütterlichen Blut nachgewiesen werden. Weiterhin ist Zink wichtig für die antibakterielle Aktivität des Fruchtwassers (FW). In Ländern mit durch Zinkmangel verursachtem Kleinwuchs ist auch die Rate der Säuglinge mit neurologischen Schäden stark erhöht. Im Tierversuch kommt es bei künstlich provoziertem Zinkmangel regelmäßig zu Aborten oder schweren Mißbildungen, der Geburtsvorgang ist verzögert.

Wegen der offensichtlichen Bedeutung des Zinkstoffwechsels in der Perinatalperiode erschien die eingehende Untersuchung des Fruchtwassers wichtig, das im Gegensatz zum Serum bisher nur in geringem Umfang überprüft worden ist. Wegen der engen Korrelation zwischen Zink- und Proteinstoffwechsel mußte die Arbeit auch vergleichende Proteinmessungen umfassen. Für beide Parameter wurden typische Untersuchungsmethoden entwickelt. Zink wurde mittels Atomabsorptions-Spektrometrie bestimmt. Physikalische und chemische Interferenzen wurden ausgeschlossen und eine Verfälschung der Ergebnisse durch eingeschleppte Verunreinigungen überprüft. Die relative Standardabweichung der einzelnen Messung liegt im Konzentrationsbereich von 1 µg Zn/ml FW bei etwa 1%, die Grenzkonzentration bei etwa 0,02 µg Zn/ml FW und die Wiederfindungsrate bei linearer Eichkurve bei 92,6%. Der Zeitaufwand pro Bestimmung liegt bei wenigen Minuten, da außer eventuellem Zentrifugieren die Fruchtwasserproben vor der Messung nicht aufbereitet werden müssen. Das Verfahren ist weitgehend automatisierbar und von angelerntem Personal durchführbar.

Die Gesamteiweißbestimmung (GEW) erfolgte nach einer für Fruchtwasser modifizierten BIURET-Methode. Wegen der niedrigeren Eiweißkonzentration im FW wurde der für das Serum gültige Reaktionsansatz der Testkombination der Firma Boehringer/Mannheim entsprechend modifiziert. Die relative Standardabweichung liegt bei Proteingehalten von etwa 200 bis 300 mg/100 ml FW bei etwa 7%, die Grenzkonzentration bei etwa 50 mg GEW/100 ml FW und die Wiederfindungsrate gemessen gegen zwei Kontrollseren bei 97,3 bzw. 94,8%. Für die Untersuchungen standen weit über 600 FW-Proben zur Verfügung, wovon für die Bestimmung von Normalwerten nur Fälle mit normalem Schwangerschaftsverlauf herangezogen wurden.

Zur Klärung der Zusammenhänge wurde der Zink- und Proteingehalt des FW in Abhängigkeit vom Lebensalter der Mutter- und in Abhängigkeit von der Schwangerschaftswoche untersucht. Der Zinkgehalt ist nicht unabhängig vom Lebensalter der Mutter, wohl aber von der Schwangerschaftswoche. **Im 3. Trimenon läßt sich nachdem U-Test ein hochsignifikanter Anstieg ($P < 0,001$) von etwa 0,06 µg Zn/ml FW auf etwa 0,14 µg Zn/ml FW nachweisen.** Im Vergleich zum mittleren Trimenon verdoppeln sich somit die Mediane der Zn-FW-Werte und erreichen in der 42. SSW 0,22 µg Zn/ml FW.

Bei Proteingehalt zeigt sich eine gewisse Abhängigkeit vom Lebensalter der Mutter mit erhöhten Werten bei sehr jungen Müttern und im Bereich des 30.–34. Lebensjahres. Ein Abfall der Proteingehalte ist für das 3. Trimenon nachweisbar. Weder für Zink noch für Eiweiß lassen sich Unterschiede in Abhängigkeit vom Geschlecht der Frucht feststellen.

Bei hypotrophen Feten konnten deutlich niedrigere FW-Zink-Werte gemessen werden, worüber in einer weiteren Veröffentlichung berichtet werden wird [8]. Daraus kann geschlossen werden, daß **die Zunahme des Zinkgehaltes des FW Ausdruck einer ungestörten Schwangerschaft ist.** Umgekehrt kann ein Abfall der FW-Zn-Werte als Hinweis auf eine fetale Entwicklungsstörung dienen. So

erscheint die Bestimmung des FW-Zn-Gehaltes der Bestimmung des Eiweißgehaltes in mehrerer Hinsicht überlegen und im Gegensatz zu dieser zur Kontrolle einer ungestörten Fetalentwicklung als geeignet.

Schlüsselwörter: Fetalüberwachung, Fruchtwasser, Mangelentwicklung, Proteine, Schwangerschaft, Spurenelemente, Zink.

Résumé

L'intéret de la mesure du zinc dans le liquide amniotique. 1ère Communication: Mise au point technique et évaluation comparative du zinc et des protéines

Le zinc est un des oligo-éléments essentiels, tant par sa présence dans d'importantes enzymes que par son rôle dans la biosynthèse protéique. Son insuffisance peut être détéctée chez l'homme comme chez l'animal. L'hypoalbuminéme due à la déficience en zinc provoque le nanisme. Chez l'homme, les causes d'insuffisance en zinc sont d'origine génétique, nutritionelle, thérapeutique et pathologique. Dans la toxémie, une baisse des teneurs en albumine et en zinc du sang maternel a été observée. De plus, le zinc joue un rôle important dans l'activité antibactérienne du liquide amniotique. Dans les pays où la déficience en zinc est à l'origine d'une petitesse de la taille, le taux de lésions neurologiques chez les enfants est aussi très élevé. Expérimentalement, les animaux soumis à une restriction de zinc avortent habituellement ou leurs petits sont fortement malformés. La mise-bas est retardée. Au vu de l'intérêt apparent du métabolisme du zinc dans la période périnatale, il a donc paru important d'étudier à fond de ce point de vue le liquide amniotique ce qui n'avait pas été fait jusque là, au contraire du sérum. En raison des interrelations entre les métabolismes du zinc et des protéines, ce dernier devait être également étudié. Les méthodes adaptées à ces déterminations ont donc été mises au point. Le zinc a été mesuré par spectrophotométrie d'absorption atomique. On a pris soin d'éliminer les interférences physiques et chimiques et on s'est soucié de l'éventualité d'une contamination exogène. La déviation standard relative de chaque mesure individuelle est d'environ 1% lorsqu'on se situe dans un niveau de concentration d'environ 1 µg Zn/ml (de liquide amniotique), et la limite de détection est d'environ 0,02 µg Zn/ml. Le taux de récupération avec une courbe de calibrage linéaire est de 92,6%. Le temps nécessaire à la mesure est de quelques minutes, car, mise à part l'éventualité d'une centrifugation, aucune préparation de l'échantillon n'est nécessaire. La technique peut être largement automatisée et confiée à des techniciens. La teneur en protéines est mesurée par la méthode du biuret adaptée au liquide amniotique. En raison de la faible concentration protéique de celui-ci, les quantités de réactifs présents dans les dispositifs de Boehringer, Mannheim et calculés pour le serum, ont été modifiées. La déviation standard relative est d'environ 7% pour une concentration protéique de 200 à 300 mg/100 ml de liquide amniotique, la limite de détection à environ 50 mg/100 ml et le taux de récupération, mesuré comparativement à 2 serums de controle, 97,3 et 94,8%. Plus de 600 échantillons de liquide amniotique furent disponibles pour cette étude, parmi lesquels seuls ceux issus de grossesses normales furent utilisés pour établir les valeurs physiologiques.

Les taux de zinc et de protéines du liquide amniotique furent étudiés en fonction de l'âge de la mère et du terme de la grossesse. La teneur en zinc est sans corrélation avec l'âge maternel, mais est liée au terme. **Au cours du 3ème trimestre, le taux de zinc s.élève de manière significative (U. test $p < 0{,}001$). d.environ 0,06 µg/ml á 0,14 µg/ml.** Les valeurs médianes doublent donc du 2ème au 3ème trimestre et atteignent 0,22 µg/ml à la 42ème semaine. Le taux de protéines dépend de l'âge de la mère, dans une certaine mesure, avec des valeurs croissantes chez les mères très jeunes et entre 30 et 34 ans. Il diminue au cours du 3ème trimestre. Le sexe du foetus n'entraine aucune différence dans les teneurs en zinc ni en protéines. Des valeurs de zinc nettement plus basses ont été trouvées en présence de foetus hypotrophiques ainsi qu'on le verra dans un prochain article [8]. On peut donc conclure que **l'augmentation du taux de zinc dans le liquide amniotique traduit une grossesse normale.** A l'inverse une diminution peut servir de révélateur d'un trouble du développement. La détermination du taux de zinc apparait donc comme préférable à celle de la teneur protéique à plusieurs points de vue, et à la différence de cette dernière, peut constituer un indicateur de développement foetal normal.

Mots-clés: Eléments de trace, grossesse à risque, liquide amniotique, protéins, surveillance foetale, zinc.

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